CONTRIBUTION TO OUR KNOWLEDGE OF THE PHYSIOLOGY OF THE PANCREAS.

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Preliminary Communication.

Historical.—Claude Bernard[1] in 1848 obtained pancreatic juice by inserting a silver canula into the larger pancreatic duct in the dog through an incision in the hypochondrium. When the canula was inserted almost immediately after a meal of meat and water, the juice was found flowing along the duct, and was collected from the canula. Inserted four hours after a meal, juice flowed at a rate of two to three drops a minute from the tube. In a dog starved for twenty-four hours no juice was obtained from the duct, and only a very few drops appeared in the canula in a day. C. Bernard[2] also noted that the introduction of ether into the stomach produced soon after a flow of pancreatic juice.

Heidenhein[3] investigated the relation of the secretion of the juice to the entry of food into the stomach, and noted, *inter alia*, the increase that occurred three and seven hours after a meal.

Dolinski[4] studying the action of bodies promoting the flow of pancreatic juice, noted that secretion was produced by the introduction of acid into the duodenum. He thought also that the acid of the chyme brought about its own neutralization by inducing a flow of alkaline pancreatic juice. Collating these results with those of other pupils, Pawlow[5] concluded that the acid was the principal factor in chyme producing a reflex secretion of pancreatic juice. Pawlow had already shown the existence of fibres in the vagus, stimulation of which was followed by a flow of the

juice. Other observers [Bernstein[6], Gottlieb[7]], earlier and later, had shown that stimulation of the peripheral ends of the vagi could inhibit an already established flow. Attempting to elucidate this, Popielski[8] observed that the flow evoked by stimulation of one vagus might be inhibited by stimulation later of the same or other vagus; and further, that the flow produced by the introduction of acid into the duodenum was regularly inhibited by stimulation of the vagus. He also found that the flow following upon the introduction of 0·4% HCl into the duodenum occurred after section of the vagi and of the sympathetic trunks. From these results he concluded that the reflex centre must lie in the abdominal cavity. His attempts to localise its position were not successful. Later[9] he showed that the flow resulting from the acid occurred after section of both vagi and the splanchnic nerves, or after destruction of the spinal cord, or after extirpation of the solar plexus.

Wertheimer and Lepage[10] found that the introduction of acid into the small intestine produced a flow which became less as the injection was made nearer to the caecum.

Bayliss and Starling[11] repeated these experiments, and found that the injection of from 30 to 50 c.c. of 0·4 % HCl into a loop of jejunum, after a latent period of two minutes, produced a marked flow of pancreatic juice. This effect was still produced after section of the vagi, section of the spinal cord at the foramen magnum, destruction of the spinal cord, section of the splanchnic nerves, extirpation of the solar plexus, or any combination of these operations. On introducing acid into a loop of intestine separated from the body except for the artery and vein, a flow of pancreatic juice was evoked. The mucosa was then scraped from this loop, ground up with sand and acid, and the extract filtered. This was introduced into the jugular vein, and called forth a copious flow of pancreatic juice. Wertheimer and Lepage[12] had already shown that the injection of acid into the circulation was without effect upon the secretion of pancreatic juice. Further investigation revealed the exceeding potency of the body in the extract, to which the name of secretin was given.

In a further paper[13] Bayliss and Starling showed that secretin from the pig, squirrel, new-born kitten, monkey, man, dog, cat, frog, tortoise, salmon, dog-fish, and skate, was active upon the dog. Also they showed that secretin produced an active flow of pancreatic juice in the rabbit, cat, and monkey. They commented, therefore, on the universality of the mechanism. They also pointed out the probability of the chemical stimulus being the active one in calling forth pancreatic juice during natural digestion.

Scope of investigation.—In this investigation the effect of extracts of the mucous membrane of the intestine of numbers of the Australian fauna has been tested, while incidentally a number of observations on factors affecting the secretion was made.

Methods, etc.—The animals used were dogs and native porcupines (*Echidna hystrix*). Ether anæsthesia was used after tracheotomy, the anæsthetic being given through a Wolff's bottle. The dogs were previously narcotized with morphia hypodermically administered. The blood pressure was recorded by a mercurial manometer connected to the carotid artery. The pancreatic juice was collected through a glass canula inserted into one of the pancreatic ducts. The drops were noted with a watch and marked by a lever on the recording cylinder. The extracts were injected into the right jugular vein by means of a burette and canula inserted into the central end of the vein, and controlled by bull dog forceps. The extracts to be tested were prepared by grinding the mucous membrane (either fresh or kept under absolute alcohol) with sand. This mucosa was extracted with from 2-5 times its weight of 0·4 % hydrochloric acid from 30 minutes to 16 hours. The extract was boiled, rendered faintly alkaline with soda, then just acid with acetic acid and filtered. The filtrate was collected in sterile flasks, which were plugged with wool while hot, and then boiled for five minutes. These will keep sterile and active. If this is not done the extracts putrefy, and the secretin disappears in from 5-10 days. In one case a badly smelling extract made seven days before was filtered

and tested. It caused a rapid secretion, though it had stood in the laboratory at 17° C. in a covered beaker.

The sterilized extracts keep for several weeks. Active secretion was produced by an extract from a dog made forty-two days previously, and with that of a cat made thirty-seven days before use. The rapid deterioration noted by May[14] has not been observed, though the extracts used have been kept at temperatures from 3°-20° C. to test the effect of temperature. May stated that the extracts from the mucosa ceased to contain secretin in two days, even when kept in an ice-chest. The following table shows some results obtained :—

Animal.	Date of Preparation.	Date of testing upon a dog.	Time kept.	Resulting rate of secretion.
Dog ...	Sept. 2nd	Sept. 5th	3 days	2 drops a minute.
Dog ..	Sept. 14th	Sept. 22nd	8 days	3 drops a minute.
Dog ...	Sept. 22nd	Oct. 4th	12 days	4 drops a minute.
Dog ...	Oct. 18th	Nov. 29th	42 days	2 drops a minute.
Cat ...	Oct. 23rd	Nov. 29th	37 days	3 drops a minute.
Echidna ...	Oct. 11th	Oct. 18th	7 days	4 drops a minute.
Ibis* ...	Sept. 27th	Oct. 4th	7 days	2 drops a minute.

This difference from May seems difficult to explain, but in two cases out of over fifty the absence of secretin was noticed. One extract was from a dog prepared seven days before testing, the other from an echidna made twenty-four hours previously.

Results.—As the work of Bayliss and Starling has been abundantly confirmed, there is no need to more than mention that their results with acids and extracts were confirmed. Secretion was also found to be brought about by the injection of pilocarpine into the circulation. This secretion differs from that produced by secretin in that it is abolished by the subsequent injection of atropine. That atropine does not abolish or affect the secretion called forth by secretin was shown by Camus and Gley,[15] Bayliss and Starling, and Wertheimer and Lepage.[16] Atropine inhibits

* Shot Sept. 15th. Intestine under absolute alcohol until Sept. 27th.

the secretion of pancreatic juice through pilocarpine, and if administered previously prevents the commencement.

Protocols of an experiment, Oct. 4th.—20 c.c. of ·25 % pilocarpine were injected into the jugular vein of a dog weighing 12 kgms. Three minutes after drops of juice fell at intervals of forty seconds for five minutes, when 15 c.c. of ·25 % atropine were injected. In three minutes secretion stopped. After twelve minutes 20 c.c. of pilocarpine solution ·25 % were injected into the vein. No secretion resulted from the pancreas, although secretion was very rapid from the salivary glands. Ten minutes later 16 c.c. of secretin were injected, and after 105 seconds secretion at five drops a minute started.

Pancreatic secretion evoked by secretin seems independent of the blood pressure. It occurs with considerable vigour even when the blood pressure is lowered greatly by the depressor substance in the intestinal extract or by toxic atropine doses.

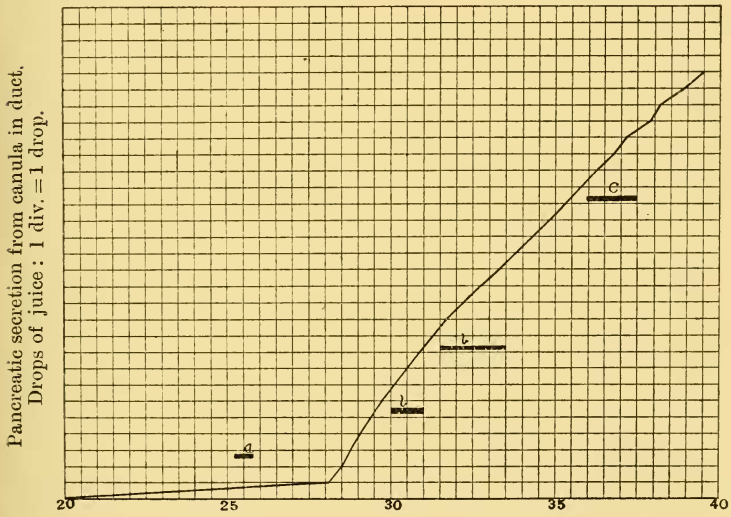
Protocol of an experiment, Sept. 14th.—There were injected 4 c.c. of an extract from the tortoise (*Chelodina longicollis*) into the jugular vein of a dog weighing 8 kgms. After 90 seconds secretion at the rate of one drop in 40 seconds was established. Five minutes later 18 c.c. of 3 % atropine sulphate solution were injected into the vein. The blood pressure fell within one minute to 5 mm. Hg., but secretion continued for nine minutes, at the rate of about one drop a minute, when the experiment ceased.

The pressure under which the juice was excreted was measured by allowing the juice to flow up a vertical tube. The fluid was raised $8\frac{1}{2}$ inches and 9 inches on two occasions The same heights were observed by filling the vertical tube to a greater height when the pressure fell to the same height as when the fluid flowed up the tube. These two methods were used in each case. The juice flowed up the tube until it remained constant. The tube was connected to the canula by a piece of rubber tubing. This allowed the vertical tube to be then raised. The juice then ran back until the height before observed was obtained. The flow of juice was very active on opening a tube connected with the canula and clamping the vertical tube, showing that active secretion was in progress. This pressure is much less than that in the arteries, the lower pressure in the carotid artery in my observations being 35 inches of blood (calculated from mercurial manometer). A like pressure was observed in the pan-

creatic duct by Pawlow when secretion was evoked by stimulating the vagus nerve.

The secretion evoked by secretin does not seem to be inhibited by stimulation of the peripheral end of the vagi, although Popielski (*supra vide*) found the flow evoked by acid in the duodenum to be inhibited by stimulation of the vagus. Neither single slowly-repeated nor rapid faradaic stimulation of the vagus produced any alteration in the rate of secretion, though marked heart inhibition and fall of blood pressure resulted. This is shown in the appended curve showing the results of faradaic

EFFECT OF VAGUS STIMULATION ON PANCREATIC SECRETION EVOKED BY SECRETIN IN THE DOG.



Time in minutes: 1 min. = 2 divisions.

(*a*) Injection of 20 c.c. secretin from the cat into right jugular vein. Vagi previously divided.

(*b*) Faradaic stimulation of peripheral and of left vagus nerve.

(*c*) Faradaic stimulation of peripheral and of right vagus nerve.

stimulation. Mechanical stimulation of the vagus was also without result.

Secretins prepared from the dog, cat, *Echidna hystrix*, wallaby (*Petrogale inornata*), tortoise (*Chelodina longicollis*), and ibis, were active on the pancreas of the dog.

On the other hand, no pancreatic juice could be obtained in the echidna. Five animals were used, and secretins from the echidna, dog, cat, and wallaby were tried. Eleven active extracts in all were introduced into the jugular veins of the echidnas, and care was taken to see that no mechanical blocking of the tube occurred. This result was surprising, but as it was tested on five animals I mention it. As opportunity arises I shall further test this question.

Properties of the pancreatic secretion obtained.—This was tested upon five occasions, three by myself and twice by Mr. J. L. Shellshear, a medical student working in the laboratory. The fluid was clear, colourless, limpid and odourless. Its specific gravity taken with a pyknometer was 1014. Its alkalinity was such that 10 c.c. required 13·2 c.c. of $\frac{N}{10}$ HCl to neutralise it. The solids were 2·9 parts in 100, and the ash was 0·69 parts. It contained a ferment setting free fatty acid from neutral fat, and another converting starch into maltose.

The secretion digested fresh fibrin, but did not affect fibrin which had been previously heated to 80° C. for thirty minutes in a water-bath. After treatment with enterokinase it rapidly digested fibrin, and coagulated egg-white.

It was further found that leucocytes contain a body like enterokinase. Whipped fibrin from a dog was washed and heated to 80° C. in a water-bath. Portions of this were then placed beneath the skin of a dog for twenty-four hours. Leucocytes were found to have penetrated the fibrin, by microscopic examination.

Series of tubes containing (*a*) heated fibrin and pancreatic juice, (*b*) fibrin impregnated with leucocytes and pancreatic juice, and (*c*) heated fibrin and activated pancreatic juice, were then arranged. The tubes were kept sterile by the addition of 2 % sodium fluoride or 2 % potassium arsenite. Three tubes were used for each set, making nine in all. The fibrin was digested in

the tubes of series (*b*) and (*c*) in from twenty-five minutes to four hours, varying with the quantity of juice. No alteration was perceived in the fibrin in tubes of series (*a*), even after weeks. The sterility of the tubes was tested with agar and gelatine plates.

Controls were also made with serum from the blood of the dog, from whom the leucocytes were obtained. No activation of the juice was observed.

These results were repeated upon five occasions, four times using sodium fluoride, and once potassium arsenite.

Protocols of two series are appended :—

A 10 c.c. 2% NaF + 0·1 c.c. pancreatice juice − 0·5 c.c. enterokinase solution [+ fibrin.

B ,, + ,, − ,, ,,

C ,, + ,, − ,, ,,

D ,, − ,, + fibrin.

E ,, − ,, − ,,

F ,, + ,, + ,,

G ,, + ,, + fibrin impregnated with [leucocytes.

H ,, + ,, + ,,

J ,, + ,, + ,,

A^1 10 c.c. 2% K_3AsO_3 + 0·25 c.c. pancreatic juice + 0·25 c.c. enterokinase soltn. [+ fibrin.

B^1 ,, + ,, + ,, ,,

C^1 ,, − ,, + ,, ,,

D^1 ,, − ,, + fibrin.

E^1 ,, + ,, + ,,

F^1 ,, + ,, + ,,

G^1 ,, + ,, + fibrin impregnated with [leucocytes.

H^1 ,, + ,, + ,,

J^1 ,, + ,, + ,,

All the tubes were kept in a water-bath at 39° C. A, B, and C were digested in two hours; G, H, J in three hours; A^1, B^1, C^1, G^1, H^1, and J^1 in twenty-five minutes; but D, E, F, D^1, E^1, and F^1 showed no change in seventy-two hours, and D, E, and F none in ten weeks. No tube yielded any culture upon agar and gelatine plates.

Summary.

(1) Secretins from the echidna, wallaby, Australian water-tortoise, and ibis are active upon the dog in causing a flow of pancreatic juice.

(2) Secretin does not appear to cause pancreatic secretion in the echidna.

(3) The flow of pancreatic juice produced by pilocarpine is inhibited by atropine, while the flow produced by secretin is not so inhibited.

(4) Stimulation of the vagus nerve does not inhibit the secretion due to secretin.

(5) The pressure under which the fluid is secreted in the pancreatic duct is equivalent to 9 inches of the juice.

(6) Pancreatic juice may be activated by leucocytes so that it acts upon proteids.

REFERENCES.

1. Bernard, C.—Leçons de Physiologie Expérimentale, Tome ii. p.198 *et seq.*
2. ——————— *Loc. cit.*, p.226.
3. Heidenhein—Hermann's Handbuch; Physiologie der Absonderungsvorgänge, s.183.
4. Dolinski—Bull. Soc. Biol. St. Petersburg, 1895, Vol. iii.
5. Pawlow—Die Arbeit der Verdauungdrüsen. Wiesbaden.
6. Bernstein—Ber. Sach. Ges. Wiss. Leipsig, 1869.
7. Gottlieb—Arch. exp. Path. und Pharm. 1895, Bd.xxxiii.
8. Popielski—Cent. f. Physiol. 1896, Bd.x.
9. ——————— Gazette de Botkin, 1900.
10. Wertheimer et Lepage—Journ. de Physiol. iii. (1901), p.335.
11. Bayliss and Starling—Journ. of Physiol. xxviii. p.325.
12. Wertheimer et Lepage—*Loc. cit.*, p.695.
13. Bayliss and Starling—Journ. of Physiol. xxix. p.174.
14. May—Journ. of Physiol. xxx. p.405.
15. Camus et Gley—C.R. Soc. Biol. Paris, 1902, p.465.
16. Wertheimer et Lepage—C.R. Soc. Biol. Paris, 1901, p.759.